2018-08-16

How does the environment affect fighting? The interaction between extrinsic fighting ability and resource value during contests.

Lane, Sarah

10.1242/jeb.187740
Journal of Experimental Biology
Company of Biologists

HOW DOES THE ENVIRONMENT AFFECT FIGHTING? THE INTERACTION BETWEEN EXTRINSIC FIGHTING ABILITY AND RESOURCE VALUE DURING CONTESTS

Running title: Environmental effects on fighting

Sarah M. Lane* & Mark Briffa

Marine biology and ecology research centre, Plymouth University, Drake Circus, Plymouth, Devon, PL4 8AA, UK.

*corresponding email: sarah.lane@plymouth.ac.uk

Summary statement

Here we demonstrate how environmental variation can affect an individual's willingness and capacity to engage in fights over indivisible resources.

Abstract

An individual's performance during a fight is influenced by a combination of their capacity and willingness to compete. While willingness to fight is known to be determined by both intrinsic and extrinsic drivers, an individual's capacity to fight is generally thought of as solely intrinsic, being driven by a host of physiological factors. However, evidence indicates that variation in fighting ability can also be generated through exposure to different environmental conditions. Environmental contributions to fighting ability may be particularly important for animals living in spatially and temporally heterogeneous habitats, in which fights can occur between rivals recently exposed to different environmental conditions. The rapidly changing environment experienced within intertidal zones, for example, means that seawater parameters, including dissolved oxygen content and temperature, can vary across small spatial and temporal scales. Here we investigate the relative importance of these extrinsic contributions to fighting ability and resource value on contest dynamics in the beadlet sea anemone *Actinia equina*. We manipulate the extrinsic fighting ability of both opponents (through dissolved oxygen concentration prior to fights) and resource value (through seawater flow rate during the fight). Our results indicate that the extrinsic fighting ability of both opponents can interact with resource value to drive escalation patterns and that extrinsic drivers can be more important in determining contest dynamics than the intrinsic traits commonly studied. Our study highlights the need to

combine data on intrinsic state and extrinsic conditions in order to gain a more holistic view of the factors driving contest behaviour.

Keywords: Animal contests; Assessment; Fighting behaviour; Hypoxia; Resource holding potential; Resource value

1. Introduction

Traditional and recent contest theory predicts that injurious fighting is more likely to occur when the potential benefits to be gained exceed the potential costs (Maynard Smith, 1973; Parker, 1974; Lane and Briffa, 2017). Operationally this means that fighting behaviour is driven by two main variables, fighting ability or resource holding potential (RHP) and resource value (RV). The costs of entering a fight will be driven by differences in RHP between the opponents (e.g. energy expended, injuries incurred) while the potential benefits to be gained from fighting will equate to the value of the contested resource (RV). Although RHP and RV have been the subject of many studies on contest dynamics (i.e. patterns of escalation and duration), most work examines either RHP (Briffa and Elwood, 2000; Dissanayake et al., 2009) or RV (Mohamed et al., 2010; Stockermans and Hardy, 2013; Palaoro et al., 2017). In reality, these factors will affect contest behaviour simultaneously and thus it is important that we understand their additive and interactive effects.

Furthermore, variation in RHP and RV can be influenced by both extrinsic and intrinsic factors. Extrinsic (or objective) sources of variation in RV (Stockermans and Hardy, 2013) derive from the absolute properties of the resource unit such as the size of a territory

64 or the number of calories in a piece of food, while intrinsic (subjective) RV reflects the
65 subjective value different individuals place on the same resource. Fights can be affected by
66 one or both of these RV components. For instance, the intensity of fights between female
67 parasitoid wasps, *Goniozus legneri*, is driven by both extrinsic (host size) and intrinsic
68 (female age) factors, with intrinsic RV having the greatest overall impact as the value of
69 finding a host increases dramatically with female age (Stockermans and Hardy, 2013).
70 Meanwhile variation in RHP is generally considered to be determined only by intrinsic
71 factors such as body size, weapon size, condition and metabolic rate, factors driven by
72 genes, development and the effects of prior contest experiences e.g. damage sustained. Yet
73 contest intensity can also be affected by rapidly fluctuating extrinsic factors such as
74 environmental conditions, particularly those expected to affect an individual's capacity for
75 performing energetically demanding aggression, (e.g. oxygen availability [Briffa and Elwood,
76 2000; Sneddon et al., 1999] and the presence of environmental toxins [Dissanayake et al.,
77 2009]). Thus variation in RHP may also be driven by extrinsic RHP components (henceforth
78 'extrinsic RHP') via their influence on physiological factors that drive fighting performance.

79 Despite the potential for extrinsic effects on RHP, contests are usually studied in
80 experimental set-ups in which environmental conditions are held constant. While this may
81 allow the effects of intrinsic RHP to be investigated, by ignoring extrinsic drivers of RHP we
82 may be overestimating the importance of these intrinsic RHP traits. Furthermore,
83 experiments in which external conditions are manipulated could allow us to test key ideas
84 about the evolution of fighting behaviour. For example, experiments designed to distinguish
85 between the assessment rules used by individuals during fights (mutual versus self-
86 assessment –Payne and Pagel, 1997; Payne, 1998) typically test for correlations between
87 some continuous measure of intrinsic RHP (e.g. body size) and contest duration. As losers

88 decide when a contest ends, contest duration should always increase with the RHP of the
89 loser, but if mutual assessment is being used there should also be a negative correlation
90 with the RHP of the winner (Taylor and Elwood, 2003; Arnott and Elwood, 2009). There are,
91 however, limits to this correlative approach (Briffa and Elwood, 2009) and manipulating
92 extrinsic RHP offers an alternative way of probing assessment rules. Providing that the
93 extrinsic RHP of each opponent can be manipulated independently, we could incorporate a
94 categorical extrinsic RHP predictor into analyses that are analogous to the correlative tests
95 currently used.

96 In nature, extrinsic sources of RHP variation may be particularly important for
97 animals living in spatially and temporally heterogeneous habitats, in which fights can occur
98 between rivals that have recently been exposed to different environmental conditions. The
99 rapidly changing environment experienced within intertidal zones, for example, means that
100 seawater parameters, including dissolved oxygen content and temperature, can vary across
101 small spatial and temporal scales. Furthermore the exposed nature, particularly on the
102 upper shore, provides motivation for conflict as individuals vie to gain suitably sheltered
103 territory before the tide goes out. Exposure to low dissolved oxygen levels (hypoxia) has
104 been shown to reduce the fighting ability of marine invertebrates, by reducing their capacity
105 to meet the energetic demands of fighting. For example, hermit crabs *Pagurus bernhardus*
106 exposed to hypoxic conditions fight with less vigour and are less likely to win fights
107 compared with crabs exposed to normoxic seawater (Briffa and Elwood, 2000). Another
108 intertidal marine invertebrate, the beadlet sea anemone *Actinia equina*, fights over limited
109 space on rocky shores, using specialised stinging structures called acrorhagi to attack rivals
110 and convince them to relinquish their territory (Williams, 1978; Brace et al., 1979; Bigger,
111 1982). Although anemones are sedentary, conflicts over territory cause individuals to move

112 across the rocks and between the microclimates created by the changing tides. Thus
113 anemones are likely to come into contact with individuals that have recently experienced
114 different levels of dissolved oxygen, and consequently differ in their extrinsic RHP.
115 Furthermore, exposure to different environmental conditions is known to drive variation in
116 extrinsic RV in *A. equina*, with individuals exposed to flowing seawater demonstrating
117 increased persistence during fights in comparison with individuals exposed to still water
118 (Palaoro et al., 2017), reflecting a higher value placed on territories that experience greater
119 flow rates. Thus, sea anemones represent an ideal system with which to simultaneously
120 investigate the effects of extrinsic drivers of fighting ability and resource value.

121 With the exception of Briffa and Elwood (2000) studies on the effects of the abiotic
122 environment on fighting have involved fights where both individuals have been subjected to
123 the same conditions during the fight. This paradigm limits our ability to determine whether
124 extrinsic variables contribute to RHP as it is not possible to separate the effects of winner
125 and loser RHP on the outcome of the contest (i.e. which individual wins). Therefore in this
126 study we manipulate the extrinsic RHP of each individual separately in order to test for the
127 potential of additive and interactive effects of both individuals' extrinsic RHPs. Furthermore,
128 we test the idea, for the first time to our knowledge, that extrinsic variation in RHP
129 (manipulated through dissolved oxygen concentration prior to fights) and RV (manipulated
130 through seawater flow rate during the fight), and the interaction between them, should
131 influence the intensity and outcome of contests. If dissolved oxygen represents an extrinsic
132 source of RHP difference, anemones exposed to higher levels of dissolved oxygen should
133 escalate more and persist for longer than those exposed to low dissolved oxygen, and
134 ultimately should win more fights. Similarly, as flow rate represents an extrinsic RV variable
135 (Palaoro et al., 2017), anemones exposed to flowing water should escalate more and persist

for longer than those exposed to still water and should defeat opponents of similar RHP. If these two factors have an interactive effect, the most intense fights are predicted to occur when both opponents are exposed to high oxygen and high flow, and the least intense fights occur when both are exposed to low oxygen and still water. Thus, the chance of victory should be greatest for focal individuals exposed to higher dissolved oxygen fighting against opponents exposed to low dissolved oxygen under high flow conditions. We also incorporate intrinsic RHP traits into our analysis in order to determine how their influence on contest behaviour may be modified by the external environment, and to determine the relative importance of intrinsic and extrinsic RHP. Finally, as described above, we use the data from this experiment to demonstrate how manipulation of extrinsic RHP can be used as an alternative means of probing assessment rules during animal contests that avoids the need for correlative analyses based on intrinsic RHP variation.

2. Materials and Methods

Animal collection and husbandry

Actinia equina (*N* = 132) of the red/brown colour morph were collected intertidally from Portwrinkle (Cornwall UK; grid reference: SX 357539) between September and December 2017 and taken back to the lab within 1-2 hours of collection. All anemones collected were visually inspected for injury and only anemones without injury were brought back to the lab. Once in the lab anemones were housed individually in plastic tanks (23 x 16 x 17.5cm) containing 700ml of filtered seawater (pumped from Mount Batten, Plymouth, UK; grid reference: SX 48715319; Average seawater quality: pH = 8 - 8.2; salinity = 34psu [HI-96822 seawater refractometer, Hanna Instruments, RI, USA]; ammonia = 0ppm; nitrite = 1ppm;

159 nitrate = 10ppm [API saltwater master test kit, API Fishcare]) along with an air stone for
160 constant aeration. Anemones were maintained at 15°c ± 0.5°c and fed ad libitum on aquaria
161 marine flakes every 2-3 days. Tank seawater was topped up daily and replaced fully every 7
162 days with fresh filtered seawater.

163

164 *Manipulating resource holding potential and resource value*

165 All anemones were given a 7-14 day acclimatisation period before they were dislodged from
166 their position in the tank and provided with stones to attach to. Anemones were then
167 randomly allocated to one of two treatments – hypoxic (H) or normoxic (N) seawater. The
168 following day, anemones allocated to the hypoxic treatment were exposed to hypoxic
169 conditions for 30 minutes prior to being introduced to an opponent. Hypoxic conditions
170 were produced by bubbling nitrogen (rather than the usual air) into the anemone's tank
171 until O_2 levels reached 30%. The oxygen levels in the tank were then kept at 30% for 30
172 minutes by covering the tank with a piece of Perspex and monitoring O_2 levels with an
173 oxygen probe (YSI Pro2030, YSI Inc., OH, USA). Normoxic individuals were maintained under
174 normal seawater conditions before the fight. In order to create a fully orthogonal design,
175 anemones were allocated into size-matched pairs (estimated visually) according to
176 treatment and assigned at random as either the focal or opponent individual (focal-
177 opponent: - H-N, N-H, H-H and N-N). As anemones rely on water flow in the wild to find
178 food, we also manipulated resource value by altering flow conditions within the fighting
179 tanks. All fights were performed in freshly aerated sea water to control for effects of oxygen
180 content during the fight itself, then in order to create a high resource value environment,
181 half of the tanks were supplied with a small water pump (L/min) (EHEIM compactON 300,

EHEIM GMbH & Co., Deizisau, Germany) (flow (F)) while the other half were not (no flow (NF)). The pump was fully submerged to eliminate the possibility that anemones under flow conditions were receiving more oxygen, and the levels of dissolved oxygen monitored in each tank type prior to the experiment to confirm this assumption. Pairs were then randomly allocated to one of these two resource value conditions in a fully orthogonal manner, resulting in a total of 8 treatment groups (H-N (F); H-N (NF); N-H(F); N-H(NF); H-H(F); H-H(NF); N-N(F); N-N(NF)).

Staging contests

Fights in *A. equina* take two forms (1) non-injurious contact of the feeding tentacles or (2) one or both anemones inflict injurious attacks using acrorhagi leaving behind acrorhagial stinging 'peels' on the opponent. These 'peels' cause localised necrosis on the recipient but are not fatal in *A. equina*. In order to stimulate agonistic behaviour, anemones were positioned such that their body columns were touching. Fights were recorded from this initial contact until one anemone (the loser) either: (a.) moved an approximate distance of one pedal disc away from its opponent (estimated visually) or (b.) retracted its tentacles completely for at least 10 minutes. If both opponents performed these retreating behaviours, the outcome of the fight was classified as a draw. Similarly, if neither individual retreated after 3 hours, the fight outcome was classed as a draw. At the end of the contest individuals were checked for the presence of acrorhagial peels, separated and returned to their tanks. If one or both anemones failed to open their tentacles within the three hour observation period, the interaction was categorised as a 'no fight' and the anemones were removed from the study. All fights were recorded using a Canon LEGRIA HF R706 High Definition Camcorder and scored blind manually for contest behaviour and duration. A total of 66 interactions were observed with an average of 8 interactions per treatment combination, see table 1 in appendix for a full breakdown of sample sizes.

Measuring intrinsic RHP traits

After the fights the minimum and maximum pedal disc diameters of each anemone were measured using callipers to the nearest 0.1mm. As pedal disc shape is elliptical, body size was then calculated for each anemone as the average of the minimum and maximum diameter (Brace and Quicke, 1986). Tissue samples from acrorhagi that had not been used in the contest were taken from each anemone using forceps, spread onto a glass slide and stained using 1% methylene blue solution (Manuel, 1988). Anemones are capable of rapidly regenerating body parts (Brockes and Kumar, 2008; Leclère and Röttinger, 2017) and thus this removal of acrorhagi only damages the animals temporarily. Nematocysts were imaged using a Leica M205 FA stereo microscope equipped with a camera (Leica DFC7000 T, Leica microsystems Ltd. CH-9435, Heerbrugg) connected to a computer. Nematocyst length was then measured blind, using point-to-point measurements in ImageJ (version 1.50i). Nematocyst length for each individual anemone was then calculated as the average length of 10 randomly selected nematocysts.

At the end of the experiment, all anemones were returned to the shore they were collected from.

Statistical analyses

We approached the analysis in two ways. First, we tested for the effects of extrinsic RHP and RV on overall contest dynamics, in order to determine how these factors would influence (i) the occurrence of fights, (ii) the occurrence of escalated fights involving injuries, (iii) for fights that escalated, the type of fight in terms of whether one or both individuals deployed their acrorhagi (attack type), and (iv) the duration of the contest. Second, we analysed the effects of extrinsic RHP and RV from the perspective of focal individuals to determine the effects of these factors on (v) the likelihood of focal individuals deploying their acrorhagi, (vi) for those focal individuals that did attack the opponent, the number of peels that they inflicted and (vii) the chance of victory for focal individuals. In analyses (i-iv) focal and

opponent extrinsic RHP conditions were combined to give an overall extrinsic RHP factor (henceforth 'combined RHP'), with three levels: both hypoxic (H-H), both normoxic (N-N) and mixed (H-N and N-H). We then analysed the effects of combined RHP and extrinsic RV (henceforth 'RV'; flow or no flow) and their interaction on the binary measures of fight occurrence, escalation, and attack type (escalated fights only) using generalised linear models (GLMs) with a binomial error distribution. To determine the effect of the same two predictors on contest duration (which was log-transformed) we used a linear model. In analyses (v to vii) we used two factors to account for the distinct extrinsic RHP conditions of focal and opponent individuals; 'focal RHP' (H or N) and 'opponent RHP' (H or N). We then used binomial GLMs to analyse the effect of these two RHP factors, and the RV factor (flow or no flow), and their interactions, on the probability that the focal anemone attacked the opponent (i.e. deployed its acrorhagi) and on the probability of victory for focal anemones. We used a GLM with a quasipoisson error distribution (accounting for overdispersion in the data) to analyse the effect of these three predictors on the number of peels the focal individual inflicted. Two measures of intrinsic RHP, relative size difference (RSD) and relative nematocyst length (RND) (both calculated as described in Rudin and Briffa (2011)) were included in the analyses as covariates. RSD was included as a covariate in all analyses while RND was only included as a covariate in analyses of escalated fights (RND has previously been shown to only be of importance for determining outcome in escalated fights – see Rudin and Briffa, 2011). In order to explore significant effects further, we performed post-hoc linear contrasts using the glht function of the R package multcomp (Hothorn et al., 2008). Finally, to examine the assessment rules used by anemones, we performed two t-tests with contest duration as the response variable and winner or loser RHP as the

explanatory variable, respectively. All analyses were carried out in R Studio v.1.0.136 (R Core Team, 2016).

3. Results

Contest dynamics

The likelihood of a fight occurring was significantly affected by combined extrinsic RHP (hypoxic, normoxic or mixed) (X^2 = 10.55, P = 0.005), with fights being less likely to occur when both individuals had been exposed to normoxic seawater (figure 1a). Fights also occurred more often under the high resource value (flowing seawater) treatment (X^2 = 4.21, P = 0.04) (figure 1b), but there was no interaction between combined extrinsic RHP and RV (X^2 = 1.93, P = 0.38).

When fights did occur, the probability of escalation was significantly influenced by an interaction between RHP and RV (X^2 = 11.97, P = 0.0025). When both anemones were evenly matched in extrinsic RHP (i.e. when the combined extrinsic RHP was either hypoxic or normoxic) escalation was more likely under high RV (flow) than under low RV (no flow) but when anemones were mismatched (combined extrinsic RHP was mixed) the opposite pattern was seen, with fights being more likely under low RV conditions (figure 2). For fights that did escalate, there was a further effect of resource value on whether single or mutual attacks occurred (X^2 = 6.80, P = 0.009). Mutual attacks occurred more often under high resource value (flow) while single attacks occurred more often under low resource value (no flow), suggesting that opponents were more likely to strike back when the contested resource was of high value (figure 3). Attack type (single or mutual attack), was not affected

by combined extrinsic RHP (X^2 = 4.38, P = 0.11) and there was no interaction between combined extrinsic RHP and RV (X^2 = 4.56, P = 0.10). Contest duration was significantly affected by the combined RHP of pairs (X^2 = 8.43, P = 0.004). Post-hoc analyses revealed that fights between pairs of hypoxic individuals (H-H) were significantly shorter than fights between normoxic individuals (N-N; P = 0.02) and mixed pairs (H-N or N-H; p = 0.02) (figure 4), but duration was not effected by RV (X^2 = 0.04, P = 0.83) and there was no interaction between combined RHP and RV (X^2 = 0.88, P = 0.58). There was no effect of relative size difference or relative nematocyst length on any of the factors analysed (table 2 in appendix).

Focal agonistic behaviour

There was no effect of focal RHP, opponent RHP, RV, or their interactions on whether or not the focal individual attacked (table 3 in appendix). Furthermore, resource value and its interactions with focal and opponent RHP had no effect on the number of peels inflicted by focal individuals in escalated fights (table 4 in appendix). However an interaction between focal RHP and opponent RHP (X^2 = 42.11, P = 0.01) indicates that focal individuals inflicted more peels on opponents when both had received the same RHP treatment (H-H or N-N), compared with pairs that had received different treatments (H-N or N-H) (figure 5). Finally, contest outcome for focal individuals was significantly affected by a three-way interaction between focal RHP, opponent RHP and RV (X^2 = 4.25, P = 0.039) (figure 6). However, this effect was lost when individuals that drew were removed from the dataset (X^2 = 1.31, P = 0.25), indicating that this interaction was driven by differences in the distribution of fights that ended in draws across treatment combinations. Under flowing seawater conditions

every fight involved a clear outcome when both opponents were pre-treated with normoxic seawater. In contrast, under still seawater conditions, every combination of focal and opponent pre-treatment yielded a proportion of contests that resulted in a draw. There was no effect of relative size difference or relative nematocyst length on any of the factors analysed (table 1 in appendix).

Assessment rules

Contest duration was significantly affected by the extrinsic RHP of losers ($t = 3.67$, $P < 0.001$) such that fights were resolved more quickly when losers had been subject to the hypoxic treatment. In contrast, the treatment of winners had no effect on contest duration ($t = 0.24$, $P = 0.811$) (figure 7).

4. Discussion

In this study we have demonstrated that contest dynamics and decisions can be significantly impacted by extrinsic sources of variation in both resource holding potential and resource value. Furthermore, our results indicate that some aspects of fighting behaviour are affected by interactions between an individual's extrinsic RHP, the extrinsic RHP of its opponent and the value of the contested resource, while others are subject only to additive effects of RHP and RV.

Fights between hypoxic individuals, where the extrinsic component of RHP had been experimentally reduced, were significantly shorter than fights in which both individuals had been pre-treated with normoxic seawater and fights in which each individual had received a

different pre-treatment (i.e. normoxic and hypoxic seawater). Similarly, fights in shore crabs, *Carcinus maenas*, have been shown to be shorter under hypoxic conditions (Sneddon et al., 2009) and hermit crabs, *P. bernhardus*, pre-exposed to hypoxic seawater fight less intensely than those exposed to normoxic seawater (Briffa and Elwood, 2000). As in the cases of these decapod crustaceans, it also appears that that exposure to hypoxia caused a reduction in RHP in *A. equina*, since exposed individuals persisted for less time. However, despite this reduced fighting ability, the amount of damage (number of peels) inflicted by focal individuals was significantly higher in hypoxic pairs than in mixed pairs, indicating that hypoxic individuals fought more aggressively but only when matched with their opponents in terms of extrinsic RHP. Similarly, encounters involving at least one hypoxic individual were significantly more likely to result in a fight than those containing two normoxic individuals. These findings suggest that contrary to expectations, individuals with reduced fighting ability had an increased motivation to fight. Similar results were found in a study of shore crabs *Carcinus maenas* in which starved and pyrene-exposed crabs fought with greater vigour and spent more time in possession of the contested resource than control individuals (Dissanayake et al., 2009). In contrast, previous studies investigating the effect of hypoxia on fighting behaviour have found that the reduction in RHP elicited by low oxygen levels leads to a decrease in competitive ability (Sneddon et al., 1999; Briffa and Elwood, 2000). Dissanayake et al. (2009) suggested that the discrepancy between these findings could be explained by the presence of a high value resource in their study which increased the motivation to fight, a possibility that also seems likely in the current study. Although 100% of interactions between hypoxic individuals resulted in a fight, the likelihood of these contests escalating to injurious fighting was dependent upon resource value. Contests between hypoxic pairs were significantly more likely to escalate when resource value was

high (i.e. in the presence of flowing water). Thus, low extrinsic RHP appears to increase the motivation to escalate but only when the contested resource is of high value.

Since we manipulated the extrinsic RHP independently for each opponent, we could also test for its effects on tactical (i.e. escalation) and strategic (i.e. giving up) decision-making. Furthermore, we were able to ask whether these effects were modified by resource value. During escalated fights, focal individuals inflicted a higher number of peels on their opponent when both individuals had experienced the same pre-treatment prior to the contest. This result was expected as a general prediction of theory is that contests should be more intense, in terms of the agonistic tactics used, when opponents are matched in RHP (e.g. Enquist and Leimar, 1983). The outcome of a contest is expected to be driven by a similar interaction between contestant RHPs, such that (regardless of whether self or mutual-assessment is being used) an individual's chance of winning should ultimately be driven by the difference between its own RHP and that of the opponent, but we did not find this result here. Rather, there was a three way interaction between resource value, the extrinsic RHP of focal individuals and the extrinsic RHP of their opponents. Furthermore, this interaction was driven primarily by the distribution of draws across the treatments, rather than by the distribution of victories and losses. Under conditions of high RV (flowing water), clear outcomes (fights in which there was a clear winner) were more likely when both individuals were of high extrinsic RHP (normoxic pre-treatment). In contrast, if resource value was low (still seawater) draws were only recorded in pairs in which the focal had low extrinsic RHP (hypoxic pre-treatment) and the opponent had high extrinsic RHP (normoxic pre-treatment). In general, our ability to interpret contests that end in draws is limited by the fact that predictions from contest theory are based on the assumption of clear outcomes. Nevertheless, Jennings et al. (2005) make the point that draws may be common

373 in nature, and might be underrepresented in datasets obtained from fights staged under
374 controlled conditions, often within a constrained space. The prevalence of draws in the
375 current data, where fights were observed under conditions that simulated natural abiotic
376 variation, support this view. Furthermore, in a previous study on *A. equina* (Lane and Briffa,
377 2017b) in which the same individuals fought twice, draws were more prevalent in the
378 second fight than in the first. Again, repeated fights, within a short time frame, are likely in
379 nature whereas in lab studies individuals often only engage in a single fight. In their study of
380 fighting fallow deer, *Dama dama*, Jennings et al. (2005) concluded that drawn encounters
381 were more likely when opponents were evenly matched in terms of RHP, which they
382 inferred from the use of specific agonistic tactics. Here, we found a different pattern, where
383 a clear outcome was more likely when both opponents had high RHP (i.e. both were pre-
384 treated with normoxic seawater) and when fights took place under conditions of high RV
385 (flowing seawater).

386 It appears then, that although extrinsic RHP contributes to the dynamics of fighting,
387 it cannot fully explain fight outcomes. On the other hand, contest outcomes were also not
388 explained by our intrinsic measures of RHP (body size and nematocyst length), which were
389 shown to differ between winners and losers in previous studies (e.g. Rudin and Briffa, 2011;
390 2012). In those studies, extrinsic components of RHP and RV were not manipulated, so it is
391 possible that in the current study the effects of these extrinsic factors have over-ridden the
392 effects of intrinsic RHP. This still leaves the question of what might have differed between
393 winners and losers in contests where both opponents had been treated identically. One
394 possibility is that winners and losers differed physiologically such that winners were best
395 able to take advantage of the normoxic conditions. In giant freshwater prawns,
396 *Macrobrachium rosenbergii*, (Brown et al., 2003) and the ectoparasitoid wasp *Eupelmus*

397 *vuilleti* (Boisseau et al., 2017), for example, winners of fights had higher resting metabolic
398 rates compared with losers. Similarly, in the damselfish, *Pomacentrus amboinensis*, winners
399 had greater aerobic scope compared with losers (Killen et al., 2014). Although the idea that
400 fighting can be energetically demanding is widely appreciated (Briffa and Sneddon, 2007)
401 and links between metabolic rate and aggressiveness have been proposed (Reale et al.,
402 2010), relatively few studies have directly measured the effects of variation in metabolic
403 rate on fight outcomes (Earley and Hsu, 2013). Although we did not measure metabolic rate
404 in the current study, our data suggest that the effect of variation in metabolism on fight
405 outcomes might be dependent on external conditions. In *C. maenas*, for example, low
406 oxygen leads to changes in the degree to which glycogen is mobilised during fights and the
407 extent to which glycogen concentration differs between winners and losers (Sneddon et al.,
408 1999). Thus, we suggest that extrinsic components of RHP, such as oxygen tension, might
409 determine the relative importance of intrinsic RHP traits (e.g. body size, weapons size,
410 energy reserves, metabolic rate, boldness).

411 What seems apparent is that, as in other marine species, dissolved oxygen (perhaps
412 in conjunction with intrinsic physiological traits) represents an extrinsic source of variation
413 in RHP for sea anemones. Since we manipulated dissolved oxygen independently for each
414 opponent there is the potential to use the two RHP levels (normoxic = high RHP; hypoxic =
415 low RHP) to probe the assessment rules used in the fights. In the case of losers, fights lasted
416 longer when they had been pre-treated with normoxic sea water, whereas the pre-
417 treatment of winners had no effect on contest duration. This pattern indicates that losers
418 give up when they cross a threshold of persistence but that this decision is not influenced by
419 the RHP of the opponent. In a previous study (Rudin and Briffa, 2011) we found an
420 analogous result based on intrinsic RHP measures in *A. equina* that also indicated the use of

421 self-assessment. In that case, however, the ability to identify an assessment rule was
422 dependent on the choice of intrinsic RHP trait used in the correlative analysis. When
423 nematocyst length was chosen as the measure of RHP, the data clearly indicated self-
424 assessment but when dry mass was used there was no correlation between contest
425 duration and either winner or loser RHP. An explanation for that discrepancy was that the
426 importance of each intrinsic RHP trait depended on the level of escalation reached during
427 the fight. Understanding how different RHP traits contribute across escalation levels in a
428 contest is important but at the same time these differences in the importance of RHP traits
429 can hinder our ability to probe assessment rules. Furthermore, by relying on correlative data
430 there is the risk that additional unmeasured variables that co-vary with an assumed
431 predictor (i.e. body size or weapon size) might drive or obscure the patterns of interest. The
432 current data, where extrinsic RHP appears to override the intrinsic traits that normally
433 predict victory, show how manipulation of fighting ability offers an alternative approach
434 that can potentially be used to clarify conclusions based on intrinsic RHP traits.

435 While many studies have investigated the effects of RHP and RV on animal contests,
436 relatively few have directly tested the interactions between these factors. An exception is
437 Stockermans and Hardy (2013), who investigated the effects of subjective (i.e. intrinsic) RV,
438 objective (i.e. extrinsic) RV and intrinsic RHP, revealing additive rather than interactive
439 effects between the RV and RHP components studied. A potential difficulty in identifying
440 interactions between RHP and RV is that intrinsic RHP components are difficult to
441 manipulate. While extrinsic sources of RHP have been manipulated previously (Sneddon et
442 al., 1999; Briffa and Elwood, 2000), this is the first study to our knowledge to vary extrinsic
443 RHP independently for each opponent in conjunction with manipulating extrinsic RV. In
444 systems where it is feasible, manipulation of extrinsic RHP may be a useful step in probing

445 or confirming contest assessment rules. Furthermore, differences in extrinsic RHP are likely
446 to be important for animals living in heterogeneous environments, especially if they come
447 into contact with individuals that have recently experienced different environmental
448 conditions. Here we have shown how the extrinsic RHP of both opponents can interact with
449 extrinsic RV and, in sea anemones, over-ride the effects of the intrinsic RHP traits that are
450 normally studied. Thus, it seems probable that fights in a natural setting are governed by a
451 set of interactions between intrinsic and extrinsic components of RHP and RV. In order to
452 fully understand the evolution of fighting behaviour, further experiments that investigate
453 the interactions between these factors will be needed.

454

455 **Acknowledgements**

456 We thank Michael Collins for laboratory assistance and Ann Torr for help collecting
457 anemones.

458 **Competing interests**

459 No competing interests declared.

460 **Funding**

461 This study was supported by a BBSRC grant awarded to M.B. (grant no. BB/M019772/1).

462 **Data availability**

463 Data will be available from the Dryad Data Repository.

464

465 **References**

Arnott, G., and Elwood, R. W. (2009) Assessment of fighting ability in animal contests. Animal Behaviour **77**, 991-1004. doi:10.1016/j.anbehav.2009.02.010

Bigger, C. H. (1982) The cellular basis of the aggressive acrorhagial response of sea anemones. *J. Morph*. **173**, 259-278.

Boisseau, R. P., Woods, H. A., and Goubault, M. (2017) The metabolic costs of fighting and host exploitation in a seed-drilling parasitic wasp. *J. Exp. Biol.* **220**, 3955-3966. doi:10.1242/jeb.160887

Brace R. C., and Quicke, L. J. (1986) Dynamics of colonization by the beadlet anemone, *Actinia equina*. *J. Mar. Biol. Assoc. UK* **66**, 21-47.

Brace, R. C., Pavey, J., and Quicke, D.L. J. (1979) Intraspecific aggression in the colour morphs of the anemone *Actinia equina*: The 'convention' governing dominance ranking. *Anim. Behav.* **27**, 553-561.

Briffa, M., and Elwood, R. W. (2000) Cumulative or sequential assessment during hermit crab fights: effects of oxygen on decision rules. *Proc. R. Soc. B* **267**, 2445-2452. doi: 10.1098/rspb.2000.1304

Briffa, M., and Elwood, R. W. (2009) Difficulties in distinguishing between mutual and self-assessment in animal contests. *Anim. Behav.* **77**, 759-762. doi:10.1016/j.anbehav.2008.11.010

Briffa, M., and Sneddon, L. U. (2007) Physiological constraints on contest behaviour. *Funct. Ecol.* **21**, 627-637. doi: 10.1111/j.1365-2435.2006.01188.x

Brockes, J.P., and Kumar, A. (2008) Comparative aspects of animal regeneration. *Annu. Rev. Cell. Dev. Biol.* **24**, 525-549. doi: 10.1146/annurev.cellbio.24.110707.175336

Brown, J.H., Ross, B., McCauley, S., Dance, S., Taylor, A. C., and Huntingford, F. A. (2003) Resting metabolic rate and social status in juvenile giant freshwater prawns, Macrobrachium rosenbergii. *Mar. Freshw. Behav. Phy.* **36**, 31-40. doi: 10.1080/1023624021000054307

Dissanayake, A., Galloway, T. S., and Jones, M. B. (2009) Physiological condition and intraspecific agonistic behaviour in *Carcinus maenas* (Crustacea: Decapoda). *J. Exp. Mar. Biol. Ecol*. **375**, 57-63. doi: 10.1016/j.jembe.2009.05.007

Earley, R. L., and Hsu, Y. (2013) Contest behaviour in fishes. In *Animal Contests* (eds ICW Hardy, M Briffa), pp. 199-227. Cambridge, UK: Cambridge Univeristy Press.

Enquist, M., and Leimar, O. (1983) Evolution of fighting behaviour: Decision rules and assessment of relative strength. *J. Theor. Biol.* **102**, 387-410.

Hothorn, T., Bretz, F., and Westfall, P. (2008) Simultaneous Inference in General Parametric Models. *Biometrical J.* **50**, 346-363.

Jennings, D. J., Gammell, M.P., Carlin, C.M., and Hayden, T. J. (2005) Win, lose or draw: a comparison of fight structure based on fight conclusion in the fallow deer. *Behaviour* **142**, 423-439.

Killen, S. S., Mitchell, M. D., Rummer, J. L., Chivers, D. P., Ferrari, M. C. O., Meekan, M. G., and McCormick, M. I. (2014) Aerobic scope predicts dominance during early life in a tropical damselfish. *Funct. Ecol.* **28**, 1367-1376. doi: 10.1111/1365-2435.12296

Lane, S. M., and Briffa, M. (2017) The price of attack: Rethinking damage costs in animal contests. *Anim. Behav.* **126**, 23-29. doi: 10.1016/j.anbehav.2017.01.015

Lane, S. M., and Briffa, M. (2017b) Boldness is for rookies: prefight boldness and fighting success in a sea anemone. *Anim. Behav.* **132**, 13-20. doi:10.1016/j.anbehav.2017.07.012

Leclère, L., and Röttinger, E. (2017) Diversity of Cnidarian muscles: Function, anatomy, development and regeneration. *Front. Cell. Dev. Biol.* **4**:157. doi: 10.3389/fcell.2016.00157

Manuel, R. L. (1988) British Anthozoa. London, UK: Academic Press

Maynard Smith, J., and Price, G. R. (1973) The logic of animal conflict. *Nature* **246**, 15-18.

Mohamed, R., Monge, J-P., and Goubault, M. (2010) Can subjective resource value affect aggressiveness and contest outcome in parasitoid wasps? *Anim. Behav.* **80**, 629-636. doi:10.1016/j.anbehav.2010.06.022

Palaoro, A. V., Velasque, M., Santos, S., and Briffa, M. (2017) How does environment influence fighting? The effects of tidal flow on resource value and fighting costs in sea anemones. *Biol. Lett.* **13**: 20170011.doi: 10.1098/rsbl.2017.0011

Parker, G. A. (1974) Assessment strategy and the evolution of fighting behaviour. *J. Theor. Biol.* **47**, 223-243.

Payne, R. J. H., and Pagel, M. (1997) Why do animals repeat displays? *Anim. Behav.* **54**, 109-119.

549 **Payne, R. J. H.** (1998) Gradually escalating fights and displays: The cumulative assessment
550 model. *Anim. Behav.* **56**, 651-662. doi:10.1006/anbe.1998.0835

551

552 **R Core Team** (2016). R: A language and environment for statistical computing. R Foundation
553 for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

554

555 **Reale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., and Montiglio, P-O.**
556 (2010) Personality and the emergence of the pace-of-life syndrome concept at the
557 population level. *Philos. T. R. Soc. B* **365**, 4051–4063. doi: 10.1098/rstb.2010.0208

558

559 **Rudin, F. S., and Briffa, M.** (2011) The logical polyp: Assessments and decisions during
560 contests in the beadlet anemone Actinia equina. *Behav. Ecol.* **22**, 1278-1285.
561 doi:10.1093/beheco/arr125

562

563 **Rudin, F. S., and Briffa, M.** (2012) Is boldness a resource-holding potential trait?
564 Fighting prowess and changes in startle response in the sea anemone *Actinia*
565 *equina*. *Proc. R. Soc. B* **279**, 1904-1910. doi:10.1098/rspb.2011.2418

566

567 **Sneddon, L. U., Taylor, A. C., and Huntingford, F. A.** (1999) Metabolic consequences of
568 agonistic behaviour: crab fights in declining oxygen tensions. *Anim. Behav.* **57**, 353–363.

Stockermans, B. C., and Hardy, I. C. W. (2013) Subjective and objective components of resource value additively increase aggression in parasitoid contests. *Biol. Lett.* **9**, 20130391. doi: 10.1098/rsbl.2013.0391

Taylor, R. W., and Elwood, R. W. (2003) The mismeasure of animal contests. *Anim. Behav.* **65**, 1195-1202. doi:10.1006/anbe.2003.2169

Williams, R. B. (1978) Some recent observations on the acrorhagi of sea anemones. *J. Mar. Biol. Assoc. UK* **58**, 787-788.

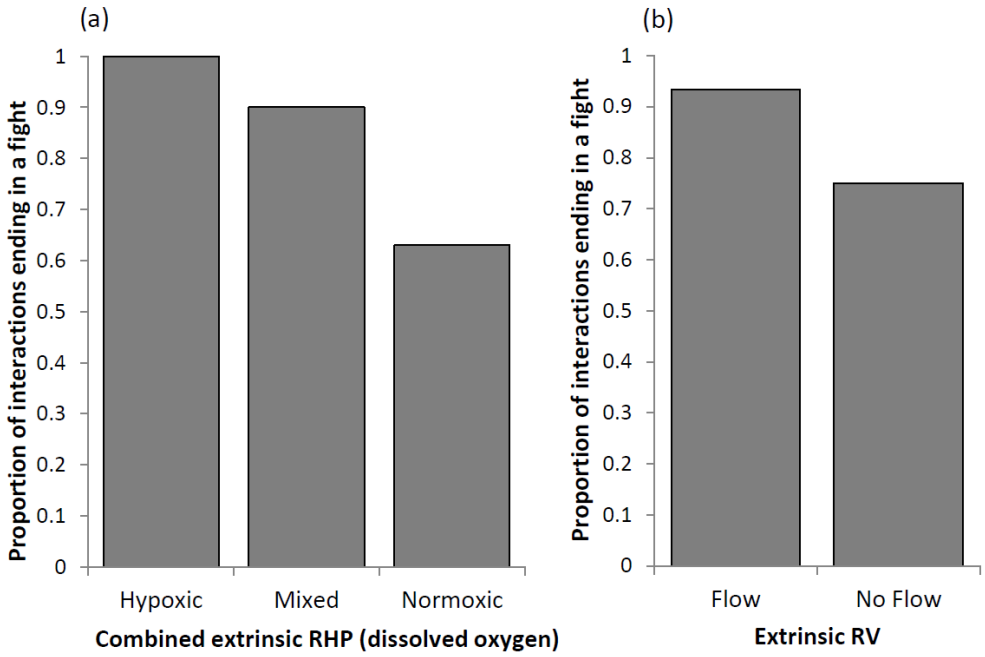

Figure 1 Effect of the (a) combined resource holding potential (RHP) of a pair of individuals (*n* = hypoxic, 16; mixed, 31; normoxic, 19) and (b) resource value (RV) (*n* = flow, 30; no flow, 36) on the likelihood of a fight occurring.

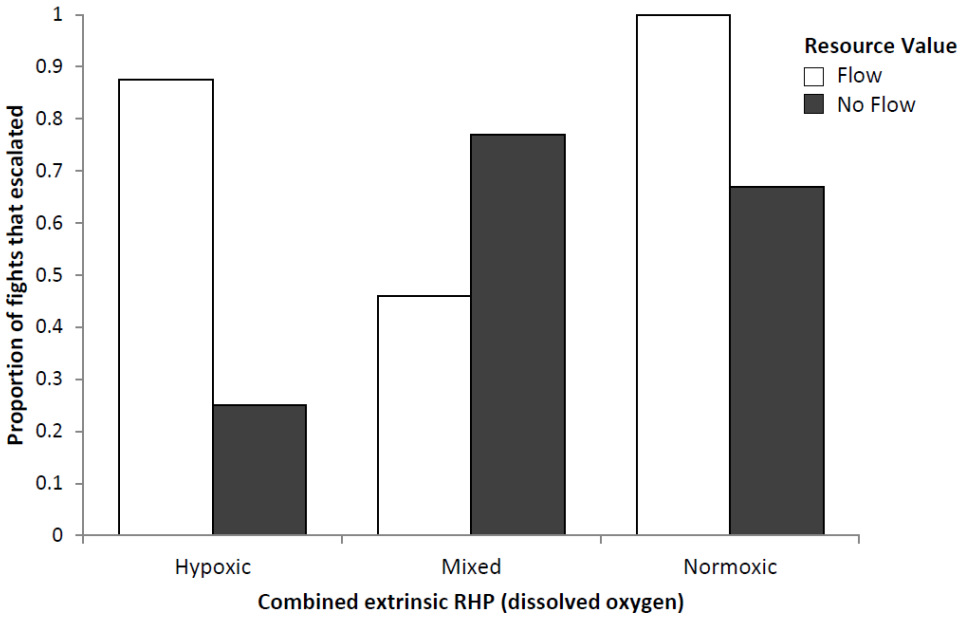

Figure 2 Interaction between combined extrinsic RHP and RV on the proportion of fights that escalated (*n* = hypoxic: - flow, 8; no flow, 8: mixed: - flow, 14; no flow, 13: normoxic: - flow, 6; no flow, 6).

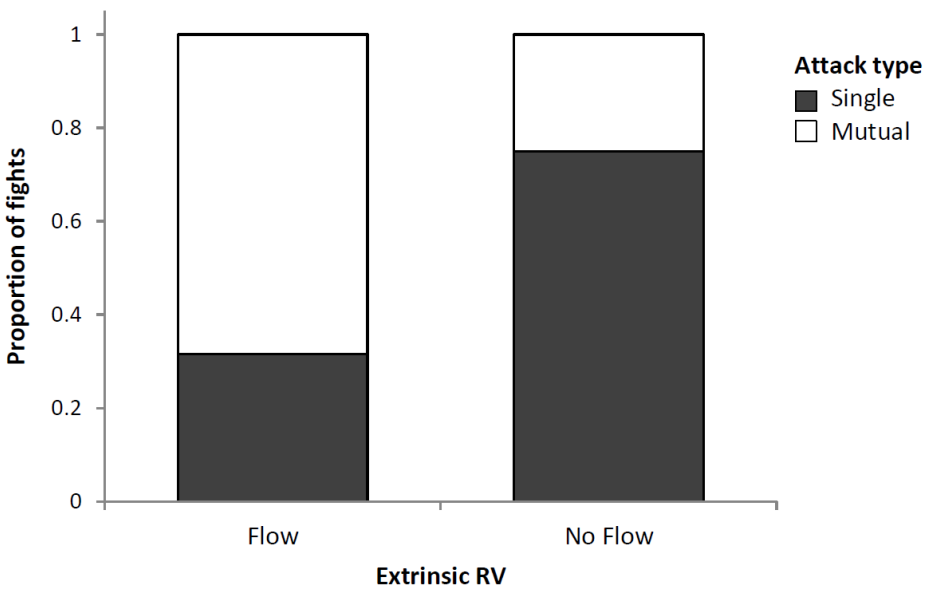

Figure 3 Effect of resource value (RV) on the type of attack exhibited during escalated fights (*n* = flow, 30; no flow, 36).

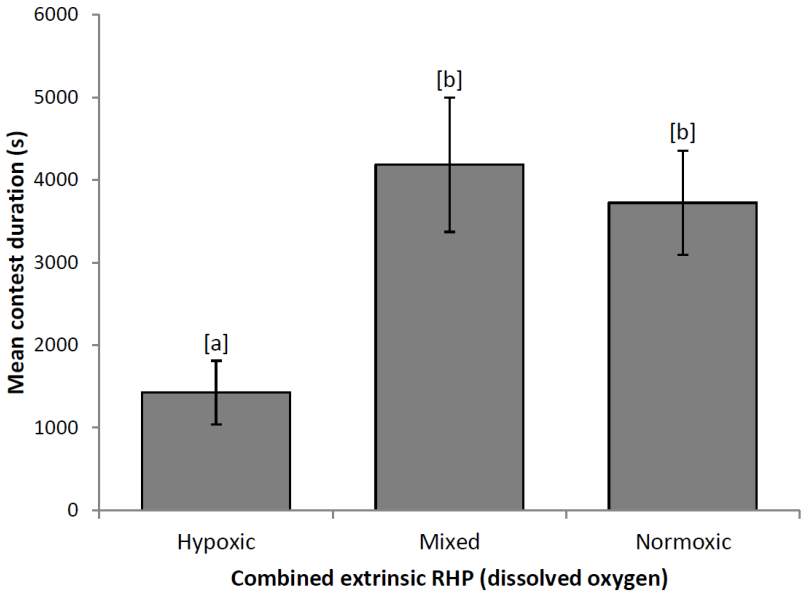

Figure 4 Effect of combined RHP on mean contest duration (seconds). Letters indicate significant differences at *P* <0.05 (*n* = hypoxic, 16; mixed, 27; normoxic, 12).

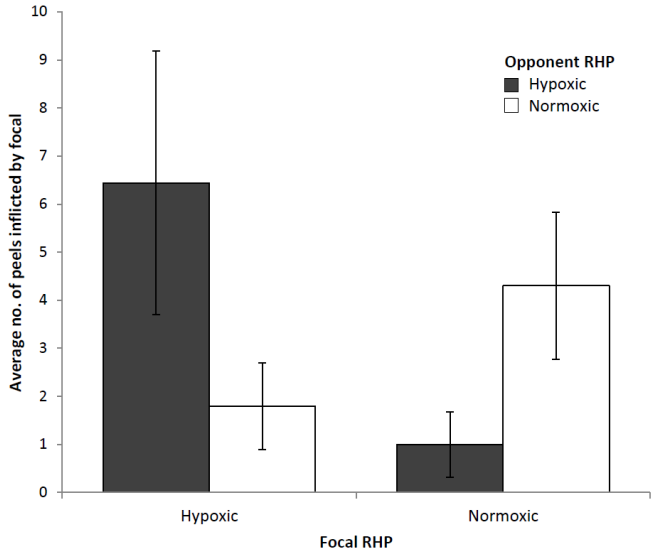

Figure 5 Interaction between focal and opponent extrinsic RHP on the mean number of peels inflicted by the focal individual (*n* = hypoxic-hypoxic, 7; hypoxic-normoxic, 6; normoxic-hypoxic, 10; normoxic-normoxic, 7).

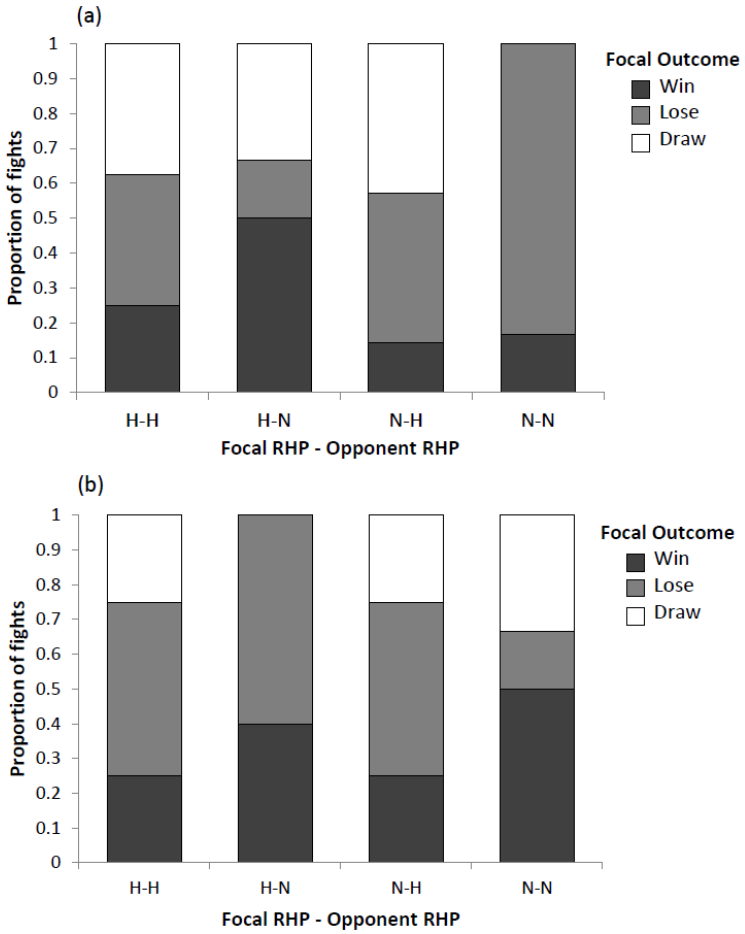

Figure 6 Interaction between focal RHP, opponent RHP and RV on focal outcome under (a) high extrinsic RV (flow) (*n* = H-H, 8; H-N, 7; N-H, 7; N-N, 6) and (b) low extrinsic RV (no flow) (*n* = H-H, 8; H-N, 5; N-H, 8; N-N, 6).

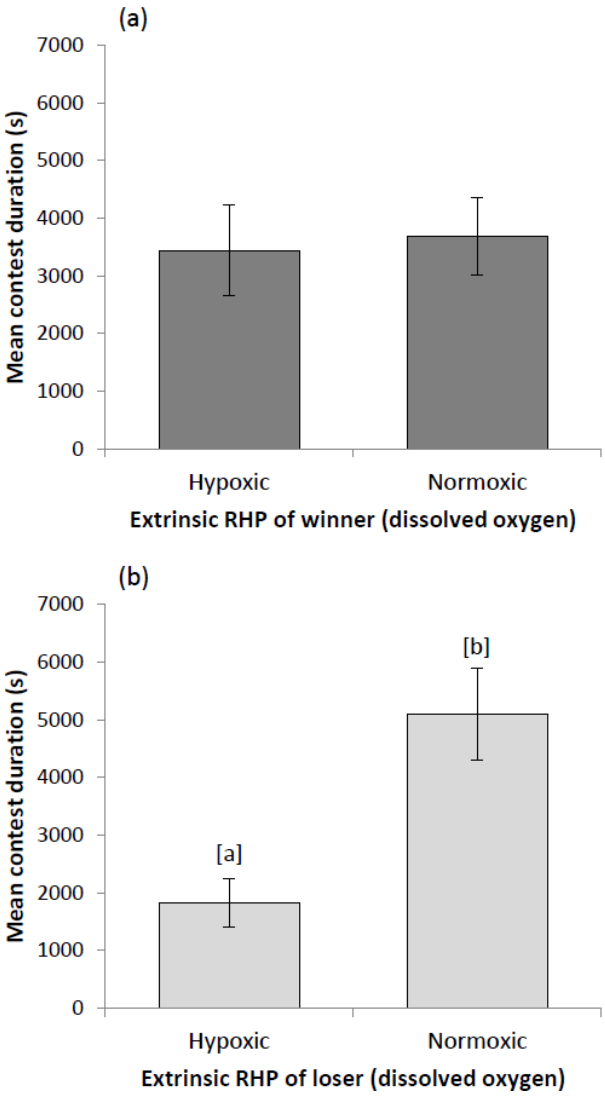

Figure 7 Effect of (a) winner (*n* = hypoxic, 23; normoxic, 17) and (b) loser (*n* = hypoxic, 19; normoxic, 21) extrinsic RHP on contest duration. Letters indicate significant differences at $P < 0.05$.

714